Myeloperoxidase (MPO) Activity Fluorometric Assay Kit

(Catalog #K745-100; 100 assays; Store kit at -20°C)

I. Introduction:

Myeloperoxidase (MPO) is a peroxidase enzyme (EC 1.11.1.7) most abundantly present in neutrophil granulocytes. It is a green hemoprotein found in neutrophils and monocytes that catalyzes the reaction of hydrogen peroxide and halide ions to form cytotoxic acids and other intermediates that play a role in the oxygen-dependent killing of tumor cells and microorganisms. Its heme pigment causes the green color in secretions rich in neutrophils, such as pus and some forms of mucus. Furthermore, it can oxidize tyrosine to a tyrosyl radical using hydrogen peroxide as an oxidizing agent. In BioVision's MPO Assay Kit, MPO catalyzes the production of NaClO from H_2O_2 and NaCl. Subsequently, NaClO will react stoichiometrically with Aminophenyl fluorescein (APF) to generate fluorescein, which can be detected at Ex/Em = 485/525 nm. The kit provides a rapid, simple, sensitive, and reliable method suitable for academic study and also high throughput assay of MPO activity. This kit can be used to detect MPO activity as low as 0.5 µU per well.

II. Kit Contents:

Component	K745-100	Cap Code	Part Number
MPO Assay Buffer	25 ml	WM	K745-100-1
MPO Substrate Stock	50 µl	Blue	K745-100-2
MPO Probe	200 µl	Red	K745-100-3
Fluorescein Standard (1 mM)	50 µl	Yellow	K745-100-4
MPO Positive Control	1 vial	Purple	K745-100-5

III. Storage and Handling:

Store the kit at -20°C and protected from light. Allow the Assay Buffer to warm to room temperature before use. Briefly centrifuge vials prior to opening. Read the entire protocol prior to performing the assay.

IV. Reagent Preparation:

MPO Substrate: Mix 4 µl MPO Substrate Stock with 700 µl Assay Buffer to prepare the MPO Substrate Solution.

MPO Positive Control: Reconstitute the MPO Positive Control with 50 µl Assay Buffer. Aliquot and store at -20°C. Use within one month. Avoid freeze thaw cycles.

V. MPO Assay Protocol:

1. **Standard Curve Preparation:** Mix 5 µl of 1 mM Fluorescein Standard with 995 µl of Assay Buffer to prepare a 5 µM Fluorescein Standard solution. Add 0, 2, 4, 6, 8, 10 µl of 5 µM Fluorescein Standard solution into a series of wells. Adjust volume to 100 µl/well with Assay Buffer to generate 0, 10, 20, 30, 40, 50 pmol/well of Fluorescein Standard. Mix well. Read the Standard Curve at Ex/Em = 485/525 nm after 5 min.
2. **Sample Preparations:** Tissues or cells can be homogenized in 4 volumes of Assay Buffer and centrifuged (13,000 x *g*, 10 min) to remove insoluble material. Serum samples can be directly diluted in the Assay Buffer. Prepare test samples of up to 50 µl/well with Assay Buffer in a 96-well plate. For white blood cells, take 2 ml of blood and lyse RBC using RBC Lysis Buffer (Cat # 5831). Incubate for 10 min. at room temperature. Centrifuge at 400 x g for 5 min. and remove the supernatant carefully. Wash the pellet with 1 ml 1X PBS. Centrifuge at 400 x g for 5 min, and remove the supernatant carefully. Lyse the pellet using 200 µl MPO Assay Buffer. Keep on ice for 10 min. Centrifuge at 10,000 x g for 10 min. to remove insoluble material. Collect the supernatant. Dilute the supernatant 10 times with MPO Assay Buffer and add 1- 10 µl of the diluted WBC lysate into a 96-well plate. Adjust the volume to 50 µl with Assay Buffer. We suggest testing several doses of your sample to make sure the readings are within the standard curve range.
3. **Positive Control (optional):**

   Add 10 µl of the reconstituted MPO Positive Control into Positive Control wells and adjust the volume to 50 µl/well with Assay Buffer.
4. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µl Reaction Mix:

   46 µl MPO Assay Buffer
   2 µl MPO Substrate Solution
   2 µl MPO Probe

   Add 50 µl of the Reaction Mix to each well containing the Positive Controls and Samples. Mix well. **(DO NOT ADD TO STANDARDS)**
5. **Sample Measurement:** Read Ex/Em = 485/525 nm R_1 at T_1. Read R_2 again at T_2 after incubating the reaction at room temperature for 30 min (or longer time if the Sample's activity is low); protect from light. The amount of RFU generated in the time interval T_2 - T_1 is ΔRFU = R_2 - R_1. It is recommended to read kinetically to choose the R_1 and R_2 within the linear range. Plot the Fluorescein Standard Curve and apply the ΔRFU to the Standard Curve to get B pmol of fluorescein (amount of fluorescein generated between T_1 and T_2 in the reaction wells). MPO activity can then be calculated:

$$\textbf{MPO Activity} = \frac{B}{(T2-T1)\times V} \times \textbf{Sample Dilution Factor} = \textbf{pmol/min/ml} = \textbf{µU/ml}$$

Where: **B** is the fluorescein amount from the Standard Curve (in pmol).
T_1 is the time of the first reading (R_1) (in min).
T_2 is the time of the second reading (R_2) (in min).
V is the pretreated sample volume added into the reaction well (in ml).

One unit is defined as the amount of Myeloperoxidase that oxidizes the substrate to yield 1.0 µmol of fluorescein per minute at room temperature.

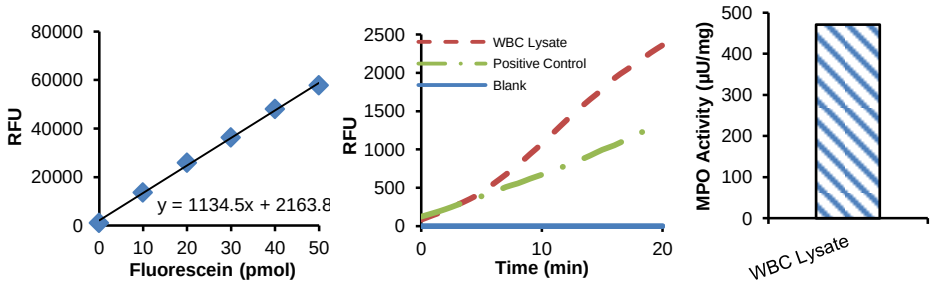

Figure: a) Fluorescein Standard Curve. b) Measurement of MPO activity in WBC lysate (0.1 µg), and MPO Positive Control (3 µl). c) MPO specific activity in WBC lysate. Assays were performed following the kit protocol.

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		